

continuation-in-part of U.S. Application Serial No. 08/179,748, filed January 7, 1994, abandoned, which in turn is a continuation-in-part of U.S. Application Serial No. 08/092,977, filed July 16, 1993, abandoned, which application is a continuation-in-part of U.S. Application Serial No. 08/017,931, filed February 12, 1993, abandoned, and is a continuation-in-part of U.S. Application Serial No. 08/292,597, filed August 18, 1994, U.S. Pat. No. 5,834,266 which in turn is a continuation-in-part of U.S. Application Serial No. 08/179,143, filed January 7, 1994, abandoned, which in turn is a continuation-in-part of U.S. Application Serial No. 08/093,499, filed Jul. 16, 1993 abandoned. The contents of each of these applications is hereby incorporated by referenced into the present disclosure.

◇ Please replace the seventh complete paragraph on page 11 with the following:

Fig. 15 depicts ligand-mediated oligomerization of chimeric proteins, showing schematically the triggering of a transcriptional initiation signal. The following abbreviations are used: Protein Kinase-C (PKC), Mitogen Activated Protein Kinase (MAPK), Diacylglycerol (DAG), Phospholipase C-gamma 1 (PLC- γ 1), Nuclear Factor of Activated T Cells-c (NFATc), and Nuclear Factor of Activated T Cells-n (NFATn).

◇ Please replace the first complete paragraph on page 45 with the following:

In some instances, one may have a target site for homologous recombination, where it is desired that a construct be integrated at a particular locus. For example, one can knock-out an endogenous gene and replace it (at the same locus or elsewhere) with the gene encoded for by the construct using materials and methods as are known in the art for homologous recombination. Alternatively, instead of providing a gene, one may modify the transcriptional initiation region of an endogenous gene to be responsive to the signal initiating domain. In such embodiments, transcription of an endogenous gene such as EPO, tPA, SOD, or the like, would be controlled by administration of the ligand. For homologous recombination, one may use either Ω or O-vectors. See, for example, Thomas and Capecchi, *Cell* (1987) 51, 503-512; Mansour, *et al.*, *Nature* (1988) 336, 348-352; and Joyner, *et al.*, *Nature* (1989) 338, 153-156.

◇ Please replace the third complete paragraph on page 47 with the following:

The ligand providing for activation of the cytoplasmic domain may then be administered as desired. Depending upon the binding affinity of the ligand, the response desired, the manner of administration, the half-life, the number of cells present, various protocols may be employed. The ligand may be administered parenterally or orally. The number of administrations will depend upon the factors described above. The ligand may be taken orally as a pill, powder, or dispersion; buccally; sublingually; injected intravascularly, intraperitoneally, subcutaneously; by inhalation, or the like. The ligand (and monomeric compound) may be formulated using conventional methods and materials well known in the art for the various routes of administration. The precise dose and particular method of administration will depend upon the above factors and be determined by the attending physician or human or animal health care provider. For the most part, the manner of administration will be determined empirically.

◇ Please replace the second complete paragraph on page 49 with the following:

In each transfection, 5 µg of expression vector, pCDL-SR (MCB 8, 466-72) (Tac-IL2 receptor –chain), encoding the chimeric receptor TAC/TAC/Z (TTZ) (*PNAS* 88, 8905-8909), was co-transfected along with various secreted alkaline phosphatase-based reporter plasmids (see map of pSXNeo/IL2 in Fig.1) in TAg Jurkat cells (a derivative of the human T-cell leukemia line Jurkat stably transfected with the SV40 large T antigen (Northrup, *et al.*, *J. Biol. Chem.* [1993])). As described in detail in the above referenced *PNAS* publication, TAC refers to the extracellular domain of the α chain of the human IL-2 receptor (the Tac antigen). Each reporter plasmid contains a multimerized oligonucleotide of the binding site for a distinct IL-2 enhancer-binding transcription factor within the context of the minimal IL-2 promoter or, alternatively, the intact IL-2 enhancer/promoter upstream of the reporter gene. After 24 hours, aliquots of cells (approximately 10⁵) were placed in microtiter wells containing log dilutions of bound anti-TAC (CD25) mAb (33B3.1; AMAC, Westbrook, ME). As a positive control and to control for transfection efficiency, ionomycin (1µm) and PMA (25 ng/ml) were added to aliquots from each transfection. After an additional 14 hour incubation, the supernatants were assayed for the alkaline phosphatase activity and these activities were expressed relative to that of the positive control samples. The addition of 1 ng/ml FK506 dropped all activity due to NFAT to background levels, demonstrating that deactivations are in the same pathway as that blocked by FK506. Each data point obtained was the average of two samples and the experiment was

performed several times with similar results. See Fig. 5. The data show that with a known extracellular receptor, one obtains an appropriate response with a reporter gene and different enhancers. Similar results were obtained when a MAb against the TcR complex (i.e. OKT3) was employed.

◇ Please replace the first complete paragraph on page 50 with the following:

Ionomycin (1 μ m) and PMA (25 ng/ml) were added to 10^5 TAg-Jurkat cells. In addition, titrations of the various drugs were added. After 5 hours the cells were lysed in mild detergent (i.e. Triton X-100) and the extracts were incubated with the β -galactosidase substrate, MUG (methyl galactosidyl umbelliferone) for 1 hour. A glycine/EDTA stop buffer was added and the extracts assayed for fluorescence. Each data point obtained was the average of two samples and the experiment was performed several times with similar results. Curiously, FK1012B appears to augment mitogen activity slightly at the highest concentration (i.e. 5 μ g/ml); however, a control experiment shows that FK1012B is not stimulatory by itself. See Fig. 6A.

A marked-up version of the amended paragraphs is provided below.

Related Applications

This application is a continuation of and claims priority to U.S. Application Serial No. 09/157,753, filed September 16, 1998, which application is a continuation of U.S. Application Serial No. 08/388,653, filed February 14, 1995, U.S. Pat. No. 5,869,337 which is a continuation-in-part of U.S. Application Serial No. 08/196,043, filed February 11, 1994, which in turn is a continuation-in-part of U.S. Application Serial No. 08/179,748, filed January 7, 1994, abandoned, which in turn is a continuation-in-part of U.S. Application Serial No. 08/092,977, filed July 16, 1993, abandoned, which application is a continuation-in-part of U.S. Application Serial No. 08/017,931, filed February 12, 1993, abandoned, and is a continuation-in-part of U.S. Application Serial No. 08/292,597, filed August 18, 1994, U.S. Pat. No. 5,834,266 which in turn is a continuation-in-part of U.S. Application Serial No. 08/179,143, filed January 7, 1994, abandoned, which in turn is a continuation-in-part of U.S. Application Serial No. 08/093,499,